

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of }
In re Application of LUTZ, *et al.* }
Filed: January 20, 2000 } Examiner: J. Kim
Serial No. 09/488,298 } Group Art Unit: 1614
For: NOVEL }
PODOPHYLLOTOXIN }
COMPOSITIONS }

}

DECLARATION OF DR. VALERY ALAKHOV UNDER RULE 1.132

I, Valery Alakhov, hereby declare that:

1. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, of both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

2. I am Vice-President R&D and Chief Scientific Officer at Supratek Pharma Inc., Montreal, Quebec, Canada H2Y 1M9. A full and accurate account of my qualifications including education, publications, titles, and awards, for example, is presented in my curriculum vitae (C.V.) as Appendix A attached hereto.

3. I have intensively studied Drug Delivery and the Medicinal Chemistry of Formulation Development, I have authored, for example, professional peer-reviewed publications including but not limited to, for example, as cited in Appendix A attached hereto.

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4. By training and experience, accordingly, I am very familiar with the current state of the art and the ongoing development toward effective formulations and delivery of anti-cancer drugs, including epipodophilotoxines and taxanes.

5. I am familiar with the statements in the present file of United States Application Serial No. 09/488,298, the specification, the claims, as well as the Amendment being filed with this Declaration.

6. I am an inventor on the above-identified pending application.

7. I confirm and attest to the fact, in view of the evidence presented herein, that I have employed d- α -tocopheryl polyethylene glycol 1000 succinate (TPGS) to solubilize podophyllotoxins and to thereby form a stable dispersion having valuable and unexpected physical and therapeutic properties.

8. I further confirm and attest to the fact, in view of the evidence presented herein, that the addition of alpha-tocopherol (*free* tocopherol) to formulations comprising Etoposide and TPGS dramatically reduces the solubility of Etoposide and lead to phase separation.

9. Generally our aqueous pharmaceutical compositions are formed which comprise Etoposide and/or Teniposide, as well as tocopherol covalently linked to a water-soluble polymer (e.g., d- α -tocopheryl polyethylene glycol 1000 succinate), wherein free tocopherol is not present.¹

¹ With regard to our specification as filed, Examples 1-3 of our patent application (pages 33-35) show example formulations of Etoposide and TPGS. No precipitation of the drug occurs in Example 1 (2 mg/ml of Etoposide and 40 mg/ml *i.e.* 4% of TPGS) when the formulation stored at room temperature for at least 36 hours. Our results further demonstrate that Etoposide is increasingly soluble in solutions of increasing concentrations of TPGS. Our results demonstrate that Etoposide is stable for longer periods of time in solutions of increasing concentration of TPGS.

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14. I am familiar with the formulations and preparation methods described by Lambert, *et al.*, in U.S. Patent No.6,458,373.

15. Formulations contemplated by Lambert, *et al.*, in U.S. Patent No.6,458,373 all require *free* tocopherol.

16. I have substantially reproduced the Etoposide formulation presented by Lambert, *et al.*, '373 Example 23 as follows:

To 4 mg of Etoposide dissolved in 1 mL of methanol/ethanol (1:1) mixture was added 50 mg of TPGS dissolved in 500 μ L of ethanol. About 300 mg of α -tocopherol (Sigma, USA) was added to the Etoposide-TPGS mixture. Then 500 μ L of 10% solution of Poloxamer 407 (50 mg total) in methanol was added to the Etoposide solution. The ethanol and methanol were removed under vacuum using a speed-vac concentrator. To the residue was added 4.5 mL of water containing 4% sorbitol and 100 mg TPGS. The mixture was shaken gently on rotator at room temperature. The final formulation became milky within one minute after dissolution and precipitation occurred within 1.5 hours after the preparation.

17. I have substantially reproduced the Etoposide formulation presented by Lambert, *et al.*, '373 Example 23 without the α -tocopherol as follows:

To 4 mg of Etoposide dissolved in 1 mL of methanol/ethanol (1:1) mixture was added 50 mg of TPGS dissolved in 500 μ L of ethanol. Then 500 μ L of 10% solution of Poloxamer 407 (50 mg total) in methanol was added to the Etoposide solution. The ethanol and methanol were removed under vacuum using a speed-vac concentrator. To the residue was added 4.5 mL of water containing 4% sorbitol and 100 mg TPGS. The mixture was shaken gently on rotator at room temperature. The final formulation was completely dissolved and produced a clear and transparent liquid. The formulation did not show any precipitation of drug when stored at room temperature for at least 24 hours.

18. I have also substantially reproduced the Etoposide formulation presented by Lambert, *et al.*, '373 Example 23, including the α -tocopherol but with higher and lower amounts

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of TPGS (Appendix B); however, due to the presence of α -tocopherol, *per se*, the drug precipitated from the formulation in each instance.

19. In view of the evidence presented herein I confirm and attest to the fact that the addition of alpha-tocopherol (free tocopherol) to formulations comprising Etoposide and TPGS dramatically reduces the solubility of Etoposide and lead to phase separation.

Date: 24.03.04

Respectfully submitted,

By:

DR. VALERY AIAKHOV

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APPENDIX A

CURRICULUM VITAE

VALERY Yu. ALAKHOV, Ph.D.

Present Position : Vice-president and CSO,
Supratek Pharma Inc.
Montreal, Quebec, Canada

Personal Data

Date and place of birth : October 7, 1957
Uzghorod, Ukraine

Citizenship : Canadian

Postal address : 531 Boul. des Prairies, Bldg. 18
Laval, Quebec H7V 1B7
Tel: (450) 686 5502, Fax: (450) 686 5504
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Education History

1986 : Ph.D., Molecular Biology,
Institute of Molecular Biology,
USSR Academy of Sciences,
Moscow, Russia

Title : Factors of Regulation of Calmodulin-
Dependent Enzymes,
Research adviser : Prof. E.S.Severin, Ph.D., Dr.Sc.

1982 : M.Sc., Organic Chemistry
Moscow Institute of Fine
Chemical Technology
Moscow, Russia

Title : Complete Amino Acid Sequence of Calmodulin
from Human Brain
Research adviser : Prof. E.S.Severin, Ph.D., Dr.Sc.

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Postdoctoral Research Training

1990 : Post-Doctoral Fellow,
Biochemistry Department,
German Cancer Research Center,
Heidelberg, Germany,
Biochemistry

1991 - 1992 : Invited Scientist,
Laboratory of Cancerology,
Biochemistry Department,
Laval University,
Quebec, Quebec, Canada
Cell Biology

Research and Academic Positions

1994-pres. : Director,
Vice-president R&D and Chief Scientist
Supratek Pharma Inc.
Montreal, Quebec, Canada

1994-pres. : Associate professor,
Immunology Research Center
Institute Armand-Frappier
University of Quebec
Laval, Quebec, Canada

1986 - 1993 : Senior Scientist (1986-1988),
Head, Laboratory of Protein Interactions (1988-1992)
Director, Department of Drug Targeting (1992-1993)
Russian Research Center of Molecular
Diagnostics and Therapy
Moscow, Russia

1982 - 1986 : Junior Research Fellow,
Laboratory of Enzyme Regulation of Cell Activity,
Institute of Molecular Biology,
USSR Academy of Sciences,
Moscow, Russia

Postdoctoral Experience : 16 years

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Teaching Experience : Scientific supervision of 12 Ph.D students and 5 M.Sc. students

Research Grants (in Canada)

| | | |
|------|---|----------|
| 1999 | : | IRAP NRC |
| 1996 | : | IRAP NRC |
| 1996 | : | IRAP NRC |
| 1994 | : | IRAP NRC |
| 1993 | : | FRSQ |
| 1991 | : | MRC |

Professional Organisations

| | | |
|------------|---|---|
| 1982-pres. | : | Russian Biochemical Society |
| 1986-pres. | : | Russian Immunological Society |
| 1992-pres. | : | American Cancer Society |
| 1995-pres | : | Canadian Society for Immunology |
| 1996-pres. | : | American Society of Pharmaceutical Sciences |
| | : | American Association for the Advancement of Science |
| 1998-pres. | : | American Chemical Society |

Areas of expertise and scientific interests

Fundamental research in the areas of drug resistance; cancer progression and genetic instability.

Applied research at the interface of formulation and material sciences, polymer chemistry, colloidal chemistry, molecular, cell-free and cellular biology.

Development of physical, chemical and biological assay systems including formulation assays (drug chemical and physical stability); ADME assays (drug serum protein interaction; drug metabolic conversion; pharmacokinetics and pharmacodynamics; toxicopharmacology, etc); biological cell-free assays (in vitro transcription/translation, phage display, gene expression profiling); biological cell-based assays (intestinal and cerebral drug transport; apoptosis;

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angiogenesis; cytotoxicity; etc.); in vivo assays (ADME analysis; tumor and inflammation models).

Industrial and management experience

Planning and supervision of R&D work both for in-house and collaborative (big pharma and biotech companies) projects.

Managing R&D budget of up to \$5M/year.

Supervising a small-to-medium (up to 40 scientists) multidisciplinary R&D group.

Supervision of the in-house work under GLP principles.

Supervision of outsourced projects under GMP compliances.

Preparation of US and Canadian IND and European CTX submissions.

Managing (on the sponsor side) the work of CRO and clinical investigators in clinical trial Phase I and Phase II setting in oncology.

Publications

More than 70 scientific publications in peer reviewed international journals; 11 issued US patents, many US and international patents are pending.

References:

Available upon request.

Selected Articles and Reviews

Reviews

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KABANOV, A.V. & ALAKHOV, V.YU. (2002) Pluronic block copolymers in drug delivery: from micellar nanocontainers to biological response modifiers, Critical Reviews: Therapeutical Drug Carrier Systems, 19 (1) 1-78.

Drug Delivery Carriers

KABANOV, A.V., SLEPNEV, V.I., KUZNETSOVA, E.L., BATRAKOVA, E.V., ALAKHOV, V.YU., MELIK-NUBAROV, N.S., SVESHNIKOV, P.G. & KABANOV, V.A. (1992) Pluronic micelles as a tool for low-molecular compound vector delivery into a cell: effect of *Staphylococcus aureus*

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SLEPNEV, V.I., KUZNETSOVA, L.E., GUBIN, A.N., BATRAKOVA, E.V., ALAKHOV, V.YU. & KABANOV, A.V. (1992) Micelles of poly(oxyethylene)-poly(oxypropylene) block copolymer (pluronic) as a tool for low-molecular compound delivery into a cell. Phosphorylation of intracellular proteins with micelle incorporated [γ -32P]ATP. *Biochemistry. Int.* 26, 587-595.

KABANOV, A.V., NAZAROVA, I.R., ASTAFIEVA, I.V., BATRAKOVA, E.V., ALAKHOV, V.YU., YAROSLAVOV, A.A., & KABANOV, V.A. (1995) Micelle formation and solubilization of fluorescent probes in poly(oxyethylene-*b*-oxypropylene-*b*-oxyethylene) solutions. *Macromolecules* 28, 7, 2303-2314.

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BATRAKOVA, E.V., LI, S., ALAKHOV, V.YU., MILLER, D.W. & KABANOV, A.V. (2002) Optimal structure requirements for pluronic block copolymers in modufying P-glycoprotein drug efflux transporter activity in BBMEC. *J. Pharmacol. Exp. Ther.*, in press.

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GEBHART, C.L. STRIADIBHATLA, S., LEMIEUX, P., ALAKHOV, V. & KABANOV, A.V. (2002) Design and formulation of polyplexes based on Pluronic-polyethyleneimine conjugates for gene transfer. *Bioconjug. Chem.* 13 (5), 937-944.

Combinatorial Biology

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POPKOV, M., SIDRAC-GHALI, S., ALAKHOV, V. & MANDEVILLE, R. (1999) Epitope-specific antibody response to HT1080 fibrosarcoma cells by mimotope immunization, *Clin. Cancer Res.* 6 (9) 3629-3635.

Cell-Free Biotechnology

KOLOSOV, M.I., ALAKHOV, V.YU., OVODOV, S.YU. & ALAKHOV, V.YU. (1992) Preparative Biosynthesis of biologically active human interleukin-2 in the continuous flow system of translation *in vitro*. *Biotechnol. and Applied Biochem.* 16, 125-133.

NAMETKIN, S.N., KOLOSOV, V.I., OVODOV, S.YU., ALEXANDROV, A.N., LEVASHOV, A.V., ALAKHOV, V.YU. & KABANOV, A.V. (1992) Cell-free translation in reversed micelles. *FEBS Lett.* 309, 3, 330-332.

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APPENDIX B

1. To 4 mg of Etoposide dissolved in 1 mL of methanol/ethanol (1:1) mixture was added 50 mg of TPGS dissolved in 500 μ L of ethanol. About 300 mg of α -tocopherol (Sigma, USA) was added to the Etoposide -TPGS mixture. Then 500 μ L of 10% solution of Poloxamer 407 (50 mg total) in methanol was added to the Etoposide solution. The ethanol and methanol were removed under vacuum using a speed-vac concentrator. To the residue was added 4.5 mL of water containing 4% sorbitol and 200 mg TPGS. The mixture was shaken gently on rotator at room temperature. The final formulation became milky within one minute after dissolution and precipitation occurred within 2 hours after the preparation.

2. To 4 mg of Etoposide dissolved in 1 mL of methanol/ethanol (1:1) mixture was added 50 mg of TPGS dissolved in 500 μ L of ethanol. About 300 mg of α -tocopherol (Sigma, USA) was added to the Etoposide -TPGS mixture. Then 500 μ L of 10% solution of Poloxamer 407 (50 mg total) in methanol was added to the Etoposide solution. The ethanol and methanol were removed under vacuum using a speed-vac concentrator. To the residue was added 4.5 mL of water containing 4% sorbitol and 50 mg TPGS. The mixture was shaken gently on rotator at room temperature. The final formulation became milky within one minute after dissolution and phase separation made in 15 minutes.

* * *